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Bacterial Dynamics in a Water Supply Reservoir
A Case Study

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Bacterial Dynamics in a Water Supply Reservoir: A Case Study

Edwin E. Geldreich, Harry D. Nash, Donald F. Spino, and Donald J. Reasoner

Adequate appraisal of the status of stored water quality and the detection of subtle ecological changes that may be occurring requires a microbiological sampling protocol that is sensitive to the three-dimensional aspects of a large body of water.

Impounded waters constitute the source water for approximately 1700 municipal supplies serving more than 55 million people, or 30.6 percent of the 180 million total population served by public water supplies.[1] Although storage and sedimentation of source water in a reservoir often improves the chemical[2] and bacterial quality of the raw water,[3,4] adverse quality changes also may be associated with these containments. Stormwater runoff from rural and urban areas may stimulate specialized microbial activity related to the biodegradation of fertilizers[4-9] and pesticides[10] or may introduce pathogens into the impoundments.[11] Inflow of poor quality source waters may introduce fecal wastes from agricultural operations[9,12,13] and municipal effluents.[14] Recreational uses of reservoirs[13,15-17] and wildlife populations[18-23] native to the watersheds may introduce additional fecal contamination into water supply impoundments. Seasonal changes in air temperature may contribute to a vertical layering of the microbial population,[24,25] and thermal loading from nuclear reactor plant discharges can stimulate increases in the heterotrophic bacterial population during periods when water temperatures are 3 to 5°C above ambient.[26] Reduced feeder stream inflows may not prevent stratification of the bacterial population.[27-29] Finally, microbial activity in the bottom sediments may bring about increased concentrations of sulfides,[30-32] iron salts,[30,31,33] manganese,[30,31,33-35] and the accumulation of oxidized organics present in waste discharges[36] or from decaying leaves and aquatic vegetation.[37]

Bacterial Population Profiles

All of these environmental factors exert pressures on the microbiological flora that result in changes in population densities and composition at the surface and alter distributions both vertically and at the water-sediment interface. The heterotrophic bacterial population of reservoir water is composed principally of gram-negative, nonspore-forming organisms.[38] The bacterial profile in water from high mountain, restricted access water supply reservoirs may be compared to high quality, groundwater-fed gravel pit lakes; i.e., predominantly achromobacteria, pseudomonads, and corynebacteria[39] with few occurrences of coliforms and other organisms of sanitary significance. Where reservoirs and impoundments are located near human habitations or where many recreational activities are permitted, increased concentrations of fecal coliforms, fecal streptococci, and other organisms associated with sanitary wastes will occur. Unless mixed by wind, water turbulence, stream inflows, or seasonal or artificial destratification, organisms of intestinal origin will concentrate in areas near the shore adjacent to recreational sites,[14,15,27,40] at the inflow zones of feeder streams,[18,41] or in areas of thermal stratification.[13,25] Dust, rain, and snowstorms may contribute to an increase in the heterotrophic bacterial populations in reservoirs and impoundments.[42] Organisms of sanitary significance, such as total coliforms, fecal coliforms, and fecal streptococci in rain or snow falling to the earth, are generally less than one organism per 100 mL.[43] Thus, the direct input of fecal bacteria due to rain and snowfall to the reservoir would be insignificant, especially when contrasted to stormwater runoff from adjacent land areas or storms along the watershed.

A vertical profile of the bacterial population in a water supply reservoir may reveal relatively uniform dispersion of organisms in shallow areas. However, in temperate zones with pronounced seasonal temperature changes, reservoirs are subject to periods of thermal stratification in the summer and winter and destratification (water turnover) periods in spring and autumn. During periods of stratification, water movement in the deep portions of reservoirs becomes restricted, generally creating a zone of maximum bacterial occurrence in the layer of water above the thermocline.[44-47] With destratification, water from the bottom and top layers mix, and a more uniform bacterial dispersion occurs throughout the reservoir until the next period of stratification develops.

The most varied microbial activity occurs at the sediment-water interface at the reservoir bottom. Sedimentation, requiring slow movement of the current and a protracted detention time, is conducive to the movement of many bacteria to the bottom strata. The densities of bacteria that become attached to sediment particles may approximate 3000 to 15 000 per mm^2 of particulate, or about one bacterium to every 70 to 300 μm^2 of available particle surface.[48] These estimates vary widely depending upon the nature of the particulate material. For example, clay bottom sediments have been found to contain more bacteria than sandy sediments.[49]

Heterotrophic bacterial activity is minimal during the winter low water temperature period. Thus, vegetative debris that was not decomposed before winter may remain essentially unchanged until the spring turnover occurs.[50] Attempts to stimulate further degradation of this organic sediment by addition of chalk ($CaCO_3$) to bottom mud have resulted in an increase in the number of ammonifying microorganisms and a decrease in the organic content of sediment in the impoundment.[51] When the input of debris from leaves, aquatic plants, decomposing algal blooms, and organic pollution discharges exceeds the biodegradation rate, a sludge or organic muck will accumulate slowly on the bottom. This organic muck becomes the habitat of a variety of bacteria whose activities include hydrocarbon oxidation, nitrification, cellulose decomposition, sulfate reduction, methane formation, and phenol degradation,[52-56] resulting in an odorous black bottom deposit.

Many of these microbial activities create taste and odor problems, particularly when hypolimnetic water must be used as a water source or during natural destratification periods.

Nonphotosynthetic pigmented bacteria often are present in reservoir water and are generally most numerous in surface layers during the summer.[57] Computer cluster analyses for grouping similar bacteria isolated from four depths in Green Lake (Wash.) revealed orange-yellow *Flavobacterium-Cytophaga* in the surface layer and yellow *Flavobacterium-Cytophaga* in the next zone.[58] No pigmented bacteria were found in the two deeper zones. Yellow-pigmented, gram-negative bacilli were the dominant organisms at the bacteria maximum of Beaver reservoir (Ark.) and appeared unable to tolerate anaerobic conditions.[45] Chromogenic bacteria have been estimated to account for 20 percent of the heterotrophic population in Lake Volta (Ghana, West Africa).[59] Since nonphotosynthetic chromogenic bacteria occasionally are found in finished water supplies, these organisms may have originated in source water and resisted removal by limited treatment processes.[60] Photosynthetic sulfur bacteria have been found to produce a red layer at the interface between the hypolimnion and epilimnion,[61] and a zone of purple sulfur bacteria was discovered at 17.4- to 21-m depths in the Green Lake reservoir.[62] Pigmented sulfur bacteria in bottom deposits of lakes appear to be associated with sludge deposits.[63,64]

Investigative Research Areas

The bacteriological quality of water supply reservoirs fluctuates as a result of a variety of factors that unbalance natural self-purification processes. Extended dry periods and extensive water withdrawals result in low water levels in reservoirs and may intensify taste, odor, and turbidity problems, as well as contribute to the deterioration of the bacteriological quality of the raw water supply.

While there has been extensive research on the heterotrophic bacterial population of reservoirs or lakes, including the isolation of highly specialized species and the identification of a variety of pigment-producing organisms, little information is available on the fate of bacterial indicators and pathogens in these waters.

Information also is needed to evaluate the effects of reservoir management practices on minimizing polluting inputs from the surrounding drainage basin and to relate the adequacy of treatment practices with fluctuating microbial populations and turbidity.

Destratification by mixing may reduce the horizontal movement of subsurface plumes of poorer bacteriological quality water within the reservoir. If this turbulence reaches the sediment-water interface, the surviving bacterial populations in the bottom material may be recirculated into the overlying waters. Recirculation of previously sedimented organic detritus may have considerable impact on the bacteriological quality of the reservoir. Mixing may result in short-term deterioration in the bacteriological quality of the reservoir water, but the long-term effects may be beneficial and result in improved water quality. Survival rates of indicator organisms and bacterial pathogens in the bottom sediment also may be significantly altered by mixing. The effects of reservoir stratification on the persistence of pathogenic and indicator bacteria in impounded waters, as these waters age or as source water pollution increases, are not clearly understood.

A number of unanswered questions pertaining to bacterial dynamics in reservoirs were investigated in a small raw water impoundment in northern Kentucky. Specifically, do seasonal turnover and stormwater runoff completely disperse or dilute the bacterial populations in the bottom material? At what fecal coliform densities in the overlying waters can *Salmonella* be detected in bottom sediments? Is there any evidence that excessively high bacterial populations develop during destratification as a result of better mixing of available nutrients and subsequent bacterial multiplication? Do excessively high bacterial populations interfere with the detection of bacterial indicator organisms?

Fig. 1. The sample locations used during the study are identified in this picture of Cruises Creek reservoir.

Reservoir Study Area

The search for a suitable water supply reservoir for this study was confined to an area within several hours driving time of the laboratory, since samples must be processed within 6 hr of collection. Inquiries were made to local water plant operators, followed by field inspections of individual sites and limited bacteriological examinations of reservoir and source water quality. Using this logistic

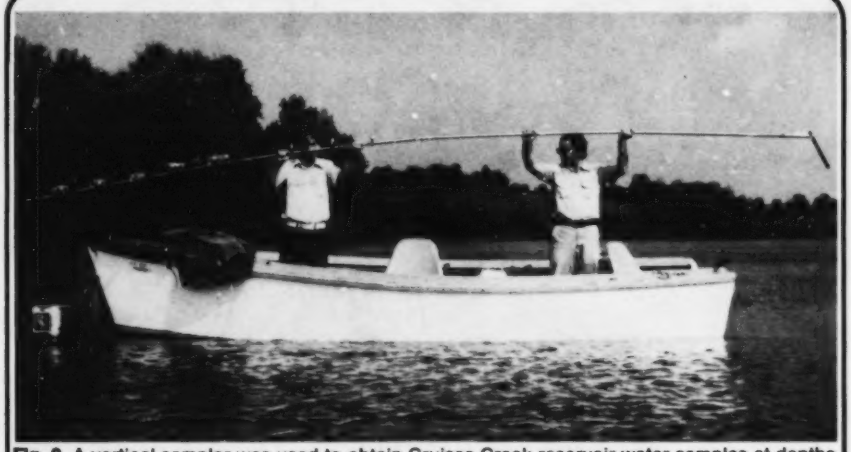


Fig. 2. A vertical sampler was used to obtain Cruises Creek reservoir water samples at depths ranging from 0.9 m (3 ft) to 9.1 m (30 ft).

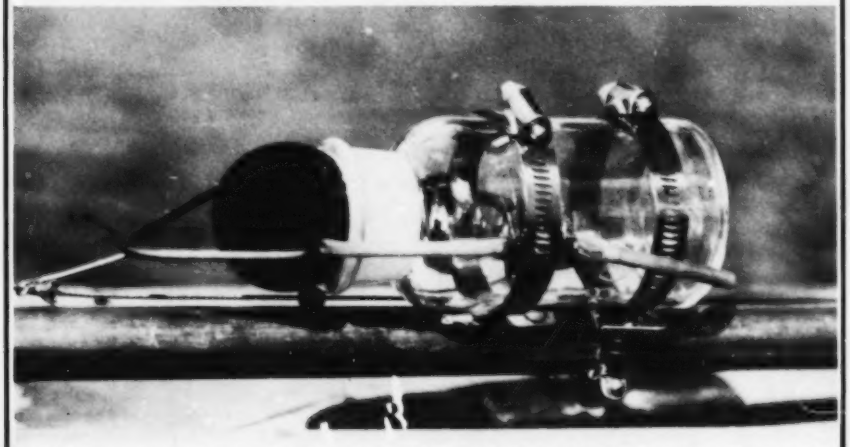


Fig. 3. Shown closeup is a sample bottle attached to the vertical water sampler, along with a cable and closure attachment.

basis, an invitation from the Walton (Ky.) Dept. of Public Works was accepted to study Walton reservoir 2, which for this study will be referred to as Cruises Creek reservoir or Walton reservoir.

Cruises Creek reservoir (Fig. 1) is located 1.6 km (1 mi) east of Walton, Ky., and is the major source of supply for the municipal water system, which serves a population of 1800. The water is treated by chemical flocculation and chlorination. The reservoir was created in 1956 by impounding Cruises Creek and several intermittent tributaries that drain approximately 312 ha (770 acres). The reservoir has a surface area of about 13 ha (32 acres), a maximum depth of 9.1 m (30 ft), and an estimated storage capacity of 3.1×10^5 m^3 (82 mil gal). An adjustable intake is used to compensate for fluctuation in the reservoir level.

The reservoir site is located in a rural area of rolling hills and is surrounded by several farms used for tobacco growing and cattle grazing. A protective fence about 100 m from the shoreline surrounds the reservoir and restricts cattle grazing, thereby providing a zone of vegetation to buffer the effects of stormwater runoff and soil erosion.

Limited recreational activity is authorized for a private fishing club of about 250 members which leases the impoundment from the water utility. The club stocks the reservoir with pike, trout, white bone, bass, catfish, crappie, and bluegill that are purchased from a source in Canada. Sanitary facilities that prevent waste discharge to the reservoir are provided, and large containers for litter control are available around the most heavily used fishing sites. Access to the reservoir by fishermen is restricted by a guard from the club during the day and by locked gate at other times.

Methods and Materials

Reservoir water. In an effort to obtain water quality measurements in this environment, new sampling methods had to be explored, evaluated, and developed as necessary. Several versions of vertical samplers have been employed to detect areas of bacterial stratification and submerged currents of differing bacteriological quality in reservoirs.[45,65,66] The vertical multiple sampler (Fig. 2) used in this study was developed by Clinton Collier* and permitted simultaneous collection of water at different depths by adjusting the location of glass sample bottles on a long pole. Each bottle was stoppered by means of a rubber ball held in place by surgical tubing (Fig. 3). The bottles were individually wrapped and sterilized prior to use. Attached to each rubber ball was a wire yoke that connected to a cable attached to a lever at the surface for control by the sample collector. Water was admitted to the sample bottles by moving the lever to unseat the ball closures. Release of the lever permitted the ball closures to reseat onto the bottle openings before retrieval of the sample bottles. The length of the sampler was adjustable from 1.5 m (5 ft) to about 9.1 m (30 ft) by inserting additional lengths of pipe and cable.

Reservoir sampling was conducted throughout a complete seasonal cycle to identify periods of natural stratification and destratification, stormwater runoff, and spring thaw over the watershed. The sampling protocol called for minimum sampling at four locations in the reservoir representing sites of inflow from feeder streams or basin drainage coves and one site near the supply intake.

Bottom sediments. Sediment deposits may provide a stable index of the general quality of the overlying water, particularly where there is great variability in the bacterial quality of the water.[67] Furthermore, where turbulent currents contact the sediment interface, surviving bacterial populations in the bottom material can be recirculated into the overlying waters. Bottom sediments in reservoirs are variable in composition, ranging from hard, rocky outcroppings to sand, gravel, or clay marl. Dredges are of limited value for some sediments when attempting to obtain representative samples from a fixed area, because of sediment looseness and buoyancy. Core sampling devices were difficult to sterilize, and the spring-loaded bacterial bottom sampler became inoperable when gritty sediment samples jammed the cutoff plate and prevented complete closure of the open end of the glass vial during recovery of the bottom sampler. Therefore, a bacteriological bottom sampler (Fig. 4) incorporating plastic collection bags was chosen for this study.[67,68] This sampler was developed for collecting bottom materials from small recreational lakes during an earlier study. Bottom samples were collected from each sam-

*Division of Shellfish Sanitation, Gulf Coast Technical Services Unit, FDA, Dauphin Island, Ala.

ple site (Fig. 1) and bags containing the samples were placed in a cooler and transported to the laboratory for processing within a 2-hr period. In the laboratory the plastic bags were cut open aseptically and all mud samples from the same site were composited in a single sterile graduate cylinder. The mud was allowed to settle until stabilized, a minimum of 1 hr, and the volume of mud and supernatant was determined. Sterile buffered dilution water was added until the liquid supernatant was equivalent in volume to the mud (1:1 dilution), and the mixture then was transferred into a wide mouth bottle containing glass beads and shaken until well homogenized.[68] Baseline bacteriological data on the sediment and overlying waters were collected.

Bacteriological analyses. Total coliform, fecal coliform, and fecal streptococcus determinations were made using membrane filter procedures and M-endo MF broth, M-FC broth, and KF streptococcus agar, respectively, as described in *Standard Methods*.[69] The general bacterial population (standard plate count) in the reservoir water was determined by using standard plate count agar and 48-hr incubation at 35°C. The Moore gauze pad technique was used to sample for salmonellae in the reservoir water by submerging the gauze pads in the water for five days. Preliminary screening of bottom sediments for salmonellae was accomplished by inoculating 30-g aliquots of settled sediment into dulcitol selenite and tetrathionate enrichment broths and incubating for 48 hr at 41.5°C.[70]

Any dulcitol selenite or tetrathionate enrichment cultures showing selenite reduction or turbidity after 24 and 48 hr were streaked onto brilliant green agar. Suspected *Salmonella* colonies were purified and characterized by the biochemical and serological tests recommended by Hajna.[71] Suspected *Salmonella* isolates were sent to the National Communicable Disease Center (Atlanta, Ga.) for confirmation of serological identification.

In situ survival studies. The in situ survival of fecal coliform, fecal streptococcus, and *Salmonella* strains isolated either from the water or bottom sediments, was determined from field site experiments using a membrane diffusion chamber.*[72] For survival experiments, the salmonellae and fecal coliforms were grown in a 6-mL volume of Hajna's GN broth at 35°C for 24 hr while a fecal streptococcus strain isolated from the reservoir was grown in 10 mL of brain heart infusion broth at 35°C for 48 hr. Cultures to be used for charging the survival chambers were centrifuged and the clear supernatant was decanted. Each cell pellet was resuspended in 10 mL of sterile phosphate buffered water and thoroughly mixed using a vortex mixer. A 1:100 dilution then was prepared in 100 mL phosphate buffered water for use as a working stock.

Survival chamber suspensions were made by preparing a 1:10 000 (10^{-4}) dilution using filtered sterilized reservoir water or phosphate buffered water as required by the particular experiment. This resulted in a cell concentration of approximately 30 000 organisms per millilitre in each survival chamber.

Reservoir water used in the suspension of selected organisms was first sterilized by passage through a 0.22-μm membrane filter and checked for sterility by inoculation of an aliquot into brain heart infusion broth. If no growth was observed after 48-hr incubation at 35°C, the filtered reservoir water was considered to be sterile.

The cell suspensions and sterilized survival chambers (previously sterilized by ethylene oxide exposure, followed by storage at 35°C for 24 hr to dissipate residual gas) were transported to the reservoir site. On location, 20 mL of a 10^{-4} dilution of the bacterial suspension in reservoir water or phosphate buffered water were added to a chamber and the suspension mixed thoroughly by pumping with the 1-mL tuberculin syringe attached to the stainless steel canula with the tygon tubing (1/32-in. ID) extension inside the chamber. After mixing, 1

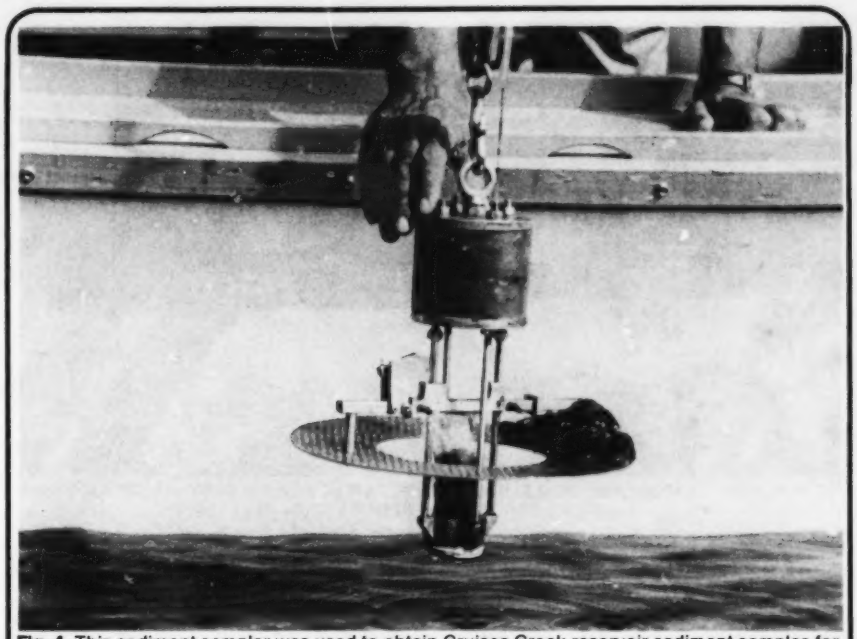


Fig. 4. This sediment sampler was used to obtain Cruises Creek reservoir sediment samples for bacteriological analyses.

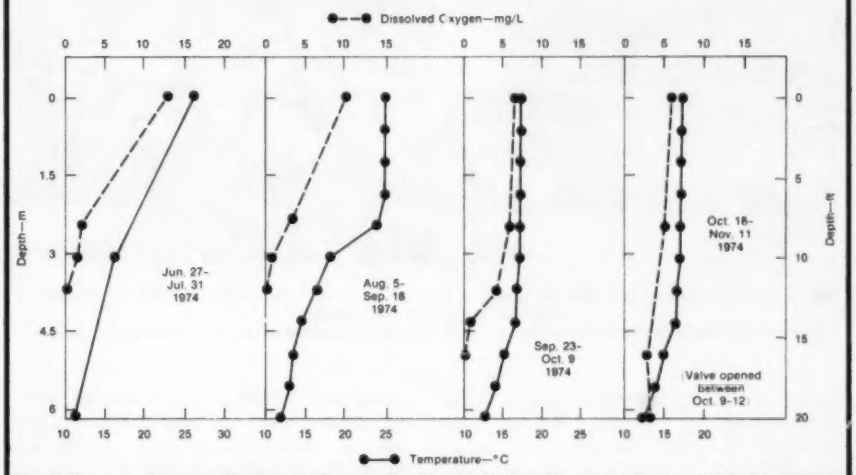


Fig. 5. Temperature and Dissolved Oxygen Profiles of Walton Reservoir During the Period From Jun. 27 to Nov. 11, 1974

*McFeters-Stuart model MSU-VME, Valley Machine and Engineering, Belgrade, Mont.

TABLE 1
Water Quality Changes in Cruises Creek Reservoir Related to Stormwater Runoff

Sampling Site	1974 Date	Dry Periods count/100 mL Total Coliforms	Dry Periods count/100 mL Fecal Coliforms	1974 Date	Stormwater Runoff Periods count/100 mL Total Coliforms	Stormwater Runoff Periods count/100 mL Fecal Coliforms
Reservoir						
R1	Mar. 25	240	34	Mar. 19	3800	450
	Mar. 27	110	14	Apr. 3	2500	1100
	Apr. 1	510	140			
R3	Mar. 25	180	28	Mar. 19	3700	480
	Mar. 27	100	12	Apr. 3	2100	870
	Apr. 1	230	80			
R4	Mar. 25	200	40	Mar. 19	3300	220
	Mar. 27	110	28	Apr. 3	3100	890
	Apr. 1	560	130			
R5	Mar. 25	220	44	Mar. 19	2500	320
	Mar. 27	96	23	Apr. 3	3500	880
	Apr. 1	250	160			
Average value		230	61		3100	650
Feeder stream						
F2	Mar. 25	400	160	Mar. 19	1900	180
	Mar. 27	960	260	Apr. 3	1400	770
	Apr. 1	2300	1600			
Average value		1200	670		1700	480

TABLE 2
*Bacterial Characterization of Cruises Creek Reservoir**

1974 Month	Surface Fecal Coliforms count/100 mL	Surface SPC count/mL	Surface Temperature °C	Mid-Depth (3–3.6 m) Fecal Coliforms count/100 mL	Mid-Depth (3–3.6 m) SPC count/mL	Mid-Depth (3–3.6 m) Temperature °C	Bottom (6.1–6.7 m) Fecal Coliforms count/100 mL	Bottom (6.1–6.7 m) SPC count/mL	Bottom (6.1–6.7 m) Temperature °C
May	26	390	21.4	55	1000	13.3	80	1200	10.3
June	5.5	1700	26.3	92	2700	16.4	230	2100	11.6
July	3.0	3200	26.6	45	16 000	16.7	190	7400	11.1
August	3.4	11 000	25.1	58	4900	17.0	100	4000	11.2
September	26	21 000	19.2	80	3700	18.2	450	8400	13.8
October	18	530	14.8	21	650	14.8	110	1900	12.7
November	6	180	9.1	4	160	11.8	11	340	11.0

*Reported as geometric mean values

TABLE 3
Effect of Reservoir Bottom Water Withdrawal

Sampling Site	October 1974 Date	Fecal Coliforms count/100 mL	SPC count/100 mL	Temperature °C	Dissolved Oxygen mg/L
Surface	2	15	480	16	
	9*	3	390	15	
	16	64	940	15	
	21	40	480	13	2.2
Mid-Depth	2	30	1000	16	0.0
33.6 m	9*	3	320	15	
(10–12 ft)	16	120	1500	15	
	21	13	540	13	2.4
Bottom	2	160	3600	12.7	0.0
6.1–6.7 m	9*	94	960	12.5	
(20–22 ft)	16	770	18 500	12.6	
	21	61	590	12.8	3.2

*Major bottom water release, Oct. 9–12, 1974

mL of suspension was withdrawn (initial sample) and placed into a screw-capped tube containing 9 mL of phosphate buffered dilution water. The tuberculin syringe was reattached to the stainless steel canula and taped in position, and a bacterial survival chamber was suspended approximately 0.3 to 0.9 m (1 to 3 ft) below the surface and at a depth of 6.1 m (20 ft) in the reservoir. A survival chamber containing sterile reservoir water also was suspended in the reservoir to serve as a control on the system integrity. At each subsequent sample collection, the connections between the TB syringe and canula were swabbed with an ethanol-soaked cotton swab, the TB syringe was removed, and a new syringe was inserted to collect a 1-mL sample. After the sample portion was withdrawn, the new TB syringe was returned to the chamber and taped in place, and the unit was resuspended at its original depth. After withdrawal of each sample portion the control survival chamber containing sterile reservoir water also was resuspended in the reservoir and was used to demonstrate continued sterility of the system in the field site. Samples collected from the bacterial survival chambers were iced and transported back to the laboratory for analysis.

In the laboratory, dilutions (10^{-5} through 10^{-7}) of the sample were made, and a 0.1-mL volume was used to inoculate each of three replicate spread plates at each dilution. Brilliant green agar, eosin methylene blue agar, and KF streptococcus agar were used for growth of salmonellae, fecal coliform, and fecal streptococcus, respectively. Possible contamination of the system was checked by inoculating standard plate count agar with 0.1-mL sample volumes from the control chambers that contained only sterile reservoir water.

Several problems with the use of the survival chambers were encountered that should be considered in future field studies. Care must be taken to avoid contamination of the test bacterial suspension. This generally occurred when the 1-mL tuberculin syringes were changed during sample collection. Apparently, contaminating bacteria were introduced through the stainless steel canula that is permanently embedded in the chamber and used to withdraw the sample. This problem was solved by swabbing the canula locking device and around the TB syringe with 70 percent ethanol prior to replacing with a new sterile syringe, pumping the syringe to mix the sample, and withdrawing the sample. In lakes or reservoirs with high fish populations, it may be desirable to modify the chambers to provide a wire mesh protector on the outside to prevent the membrane filter walls from being ruptured by fish or other aquatic animals. One such modification has been noted in the recent literature.[73] As has been observed with other kinds of survival chambers or sacs, cellulosic membrane filters tend to become colonized by microorganisms on the surface exposed to the reservoir water. This is apparent by the appearance of a "scum" on the membrane surface. Undoubtedly there could be organisms present capable of metabolizing the membrane filter material, particularly in warmer climates and during summer months. Polycarbonate filters* have been proposed as a better material for the side walls of the bacterial survival chamber and were used in a study of bacterial persistence in a thermally stressed lake.[73] However, in that study the filters eventually became colonized by epiphytic organisms.

Results and Discussion

The fecal coliform density (Table 1) in the reservoir during early spring ranged from an average of 61 organisms per 100 mL during dry periods to 650 organisms per 100 mL following stormwater runoff.

*Nuclepore Corp., Pleasanton, Calif.

The average total coliform density during the two periods ranged from 230 to 3100 organisms per 100 mL, respectively. Fecal pollution responsible for the tenfold increase in indicator organisms during or after storms originated from farm animals and indigenous wildlife on the surrounding drainage area. Rain and snowfall runoff carried the organisms into the reservoir.

Impoundment of the creek source waters results in an improvement of the bacteriological quality of the water, as was previously observed in other reservoir studies.[28,74,75] Fecal coliform levels in feeder stream F2 averaged 670 per 100 mL during dry periods and 480 per 100 mL during stormwater runoff periods (Table 1). The decrease in fecal coliform concentration in the feeder stream water during stormwater runoff periods probably reflects a dilution effect. Comparison of levels of fecal coliforms in feeder streams and reservoirs shows that feeder stream concentrations were generally tenfold higher than the reservoir concentrations. During early spring dry periods, fecal contamination introduced from the feeder creeks was diluted in the impoundment through sedimentation and dispersion to a concentration of approximately 10 percent of initial values. However, stormwater runoff discharged into the small reservoir temporarily reversed the reduction of organisms, and reservoir fecal coliform densities temporarily increased by about 1 log.

During the seven-month period of May through November 1974, only five rain periods lasted six days or longer. By late June one feeder creek was dry and water flow in the other two had all but ceased. With all data from samples that were collected within 48 hr after these five extended rainstorms eliminated, the remainder of the bacteriological data were considered to be a study of surface water in extended storage (Table 2). During July, stratification (as noted by the standard plate count density) did occur at a mid-depth of about 3 to 3.6 m (10–12 ft) and correlated with the formation of a thermocline (Fig. 5). During the period from August 5 to September 18, the thermocline became fully developed. Increased bacterial densities in the bottom waters during July, August, and September were related to natural siltation in the impoundment. Throughout the summer, a slow release of bottom water took place and a major drawoff of bottom water occurred from Oct. 9–12, 1974. The drawoff decreased the pool of septic water in the bottom of the reservoir and was a normal operating procedure intended to prevent the septic bottom water from entering the treatment plant. The rapid release of septic bottom water accelerated complete destratification of the reservoir as shown in Fig. 5. The data in Table 3 indicate that a drastic shift in

TABLE 4

Effect of Copper Sulfate Additions on Bacterial Concentrations in Cruises Creek Reservoir

Surface				Mid-Depth*			
1974 Date	Fecal Coliforms count/ 100 mL	SPC count/ mL	Temperature °C	1974 Date	Fecal Coliforms count/ 100 mL	SPC count/ mL	Temperature °C
Jun. 4	41	330		Jun. 4	680	2700	
11†	53	730	24.0	11	350	3300	15.1
13	3	22 000	24.6	13	180	7300	17.0
18	1	1200	22.0	18	87	1300	15.0
20	2	2700	23.7	20	5	3400	18.6
Jul. 1	3	500	24.8	Jul. 1	280	13 000	18.0
9‡	110	5100	28.5	9	86	46 000	17.8
18	< 1	50 000	27.3	18	144	31 000	15.3
22	2	27 000	26.2	22	48	25 000	16.5
24	12	4700	26.5	24	50	19 000	16.0
29	< 1	2800	27.0	29	18	11 000	16.0
31	1	470	26.0	31	11	5100	17.0
Aug. 5§	3	6200	24.5	Aug. 5	19	4000	15.7
7	< 1	13 000	24.9	7	16	4800	17.1
14	1	15 000	25.8	14	25	2000	16.5
19	35	25 000	25.5	19	2400	14 000	18.7
Sep. 16	103	3900		Sep. 16	580	5700	
18	31	2700	21.0	18	240	7200	18.3
23	18	2400	18.5	23	49	4100	18.2
25	8	900	18.0	25	11	1300	18.0

*3.0–3.6 m (10–12 ft)
†Copper sulfate treatment June 12
‡Copper sulfate treatment July 9
§Copper sulfate treatment August 6

TABLE 5

Bacterial Indicator Relationships for Cruises Creek Reservoir During January–February 1974*

Bacterial Indicator	Reservoir†					Feeder Streams†		
	R1	R2	R3	R4	R5	F1	F2	F3
Total coliforms/100 mL	740	1100	1300	1900	640	1600	1700	1600
Fecal coliforms/100 mL	83	90	58	110	69	140	210	160
Fecal coliforms—percent	11.2	8.2	4.5	5.8	10.8	8.8	12.4	10.4
Fecal streptococci/100 mL	450	400	350	490	330	170	910	100
FC–FS ratio	0.18	0.23	0.17	0.23	0.21	0.82	0.23	1.60

*Average of ten samples for each station
†See Fig. 1 for site locations

TABLE 6

Salmonella *Occurrences in Reservoir and Source Waters*

1974 Study Period Date	Source	Overlying Water Quality		Salmonella Occurrence	
		SPC* count/mL	Fecal Coliforms* count/ 100 mL	Water	Mud
Jan. 15–Feb. 20	F1†	1800	140	*S. give* (Jan. 24) *S. give* (Jan. 28)	*S. give* (Jan. 15) *S. species*‡ (Feb. 2)
	F2†	6400	120	*S. bern* (Jan. 24) *S. typhimurium* (Jan. 28)	*S. bern* (Jan. 15) *S. species* (Feb. 2)
	F3†	3500	120	*S. give* (Jan. 24) *S. give* (Jan. 28)	none detected
Oct. 7–Nov. 25	F1†	83 000	500	none detected	none detected
	F2†	96 000	400	none detected	none detected
	F3†	150 000	1000	*S. species* (Oct. 7) *S. species* (Oct. 30)	none detected
Aug. 14–Nov. 26	A§	1900	100	none detected	none detected
	B§	4000	230	none detected	none detected
	C§	3300	160	none detected	none detected
	D§	890	160	none detected	none detected
	E§	900	100	none detected	none detected
	G§	900	80	none detected	none detected

*Geometric means
†Feeder streams
‡*Salmonella* species
§Reservoir site

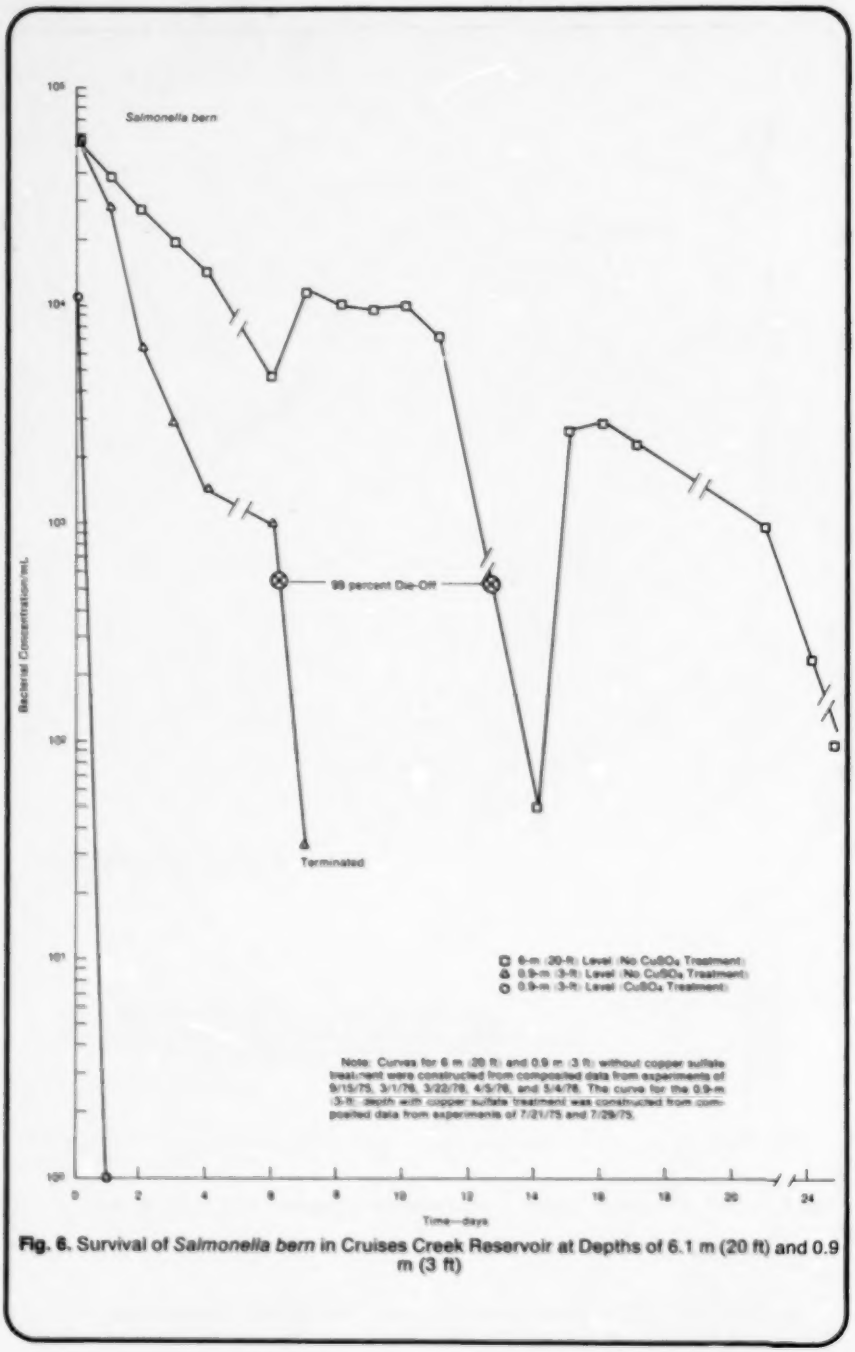

Salmonella bern
99 percent Die-Off
Terminated
Bacterial Concentration/mL
Time—days
Note: Curves for 6 m (20 ft) and 0.9 m (3 ft) without copper sulfate treatment were constructed from composited data from experiments of 9/15/75, 3/1/76, 3/22/76, 4/5/76, and 5/4/76. The curve for the 0.9-m (3-ft) depth with copper sulfate treatment was constructed from composited data from experiments of 7/21/75 and 7/29/75.

Fig. 6. Survival of *Salmonella bern* in Cruises Creek Reservoir at Depths of 6.1 m (20 ft) and 0.9 m (3 ft)

bacterial concentration occurred at mid and bottom points for samples collected on Oct. 9 and Oct. 21, 1974, and that this shift correlated directly with the accelerated destratification.

Algal blooms developed in the impoundment early in the summer. To control the algal blooms and to reduce taste and odor problems in the water supply, the reservoir was treated with copper sulfate on June 12, July 9, and August 6. The immediate bacteriological effect was most noticeable in data from surface samples (Table 4). After each application of copper sulfate, the fecal coliform density declined and the standard plate count density increased sharply, both at the surface and at mid-depth. Whether growth of the standard plate count population was stimulated by a critical concentration of the heavy metal ion (Cu^{++}) or by release of nutrients as the algal cells decomposed can only be speculated. The availability of additional nutrients for bacterial growth from the algae was probably the most significant factor. The fecal coliform and standard plate count densities in the septic bottom waters were not affected (these data are not shown).

To determine the sources of fecal pollution entering the reservoir, ten samples were collected at each of five locations around the periphery of the reservoir and at sites on three small creeks that feed the reservoir. Table 5 shows representative data collected during January–February 1974, corresponding to the period of general winter precipitation producing maximum creek flow and runoff into the reservoir. All reservoir sites and one feeder stream (F2) had fecal coliform to fecal streptococcus (FC–FS) ratios ranging from 0.17 to 0.23; feeder streams F1 and F3 had FC–FS ratios of 0.82 and 1.60, respectively. FC–FS ratios during late autumn were similar, indicating that fecal pollution reaching the reservoir originated from farm animals and wildlife on the watershed.[12,43,76] The fecal coliform portion of the total coliform population ranged from 8.8 to 12.4 percent in the feeder streams and from 4.5 to 11.2 percent for all stations in the reservoir. The uniform quality (as measured by bacterial parameters) of the surface water found at the reservoir shoreline sites and in the feeder streams during dry and wet periods probably reflects both the rural character of the drainage basin and the small size of the impoundment.

Four times during the winter study period (January–February), samples of bottom mud (sediment) at 30 g per sample and the overlying water at sites F1, F2, and F3 and at six reservoir locations were examined for *Salmonella* (Table 6). Salmonellae were detected in the water from each of the feeder streams and in only two of the bottom muds (F1 and F2). During the summer period, feeder stream water could not be examined for *Salmonella* because a long dry period had dried the feeder streams or reduced them to intermittent pools of water. In October–November, following early autumn rains, mud and water samples from the same sampling sites were examined for *Salmonella*. Salmonellae were detected twice in the overlying water from only one feeder stream; all other samples were negative for *Salmonella*.

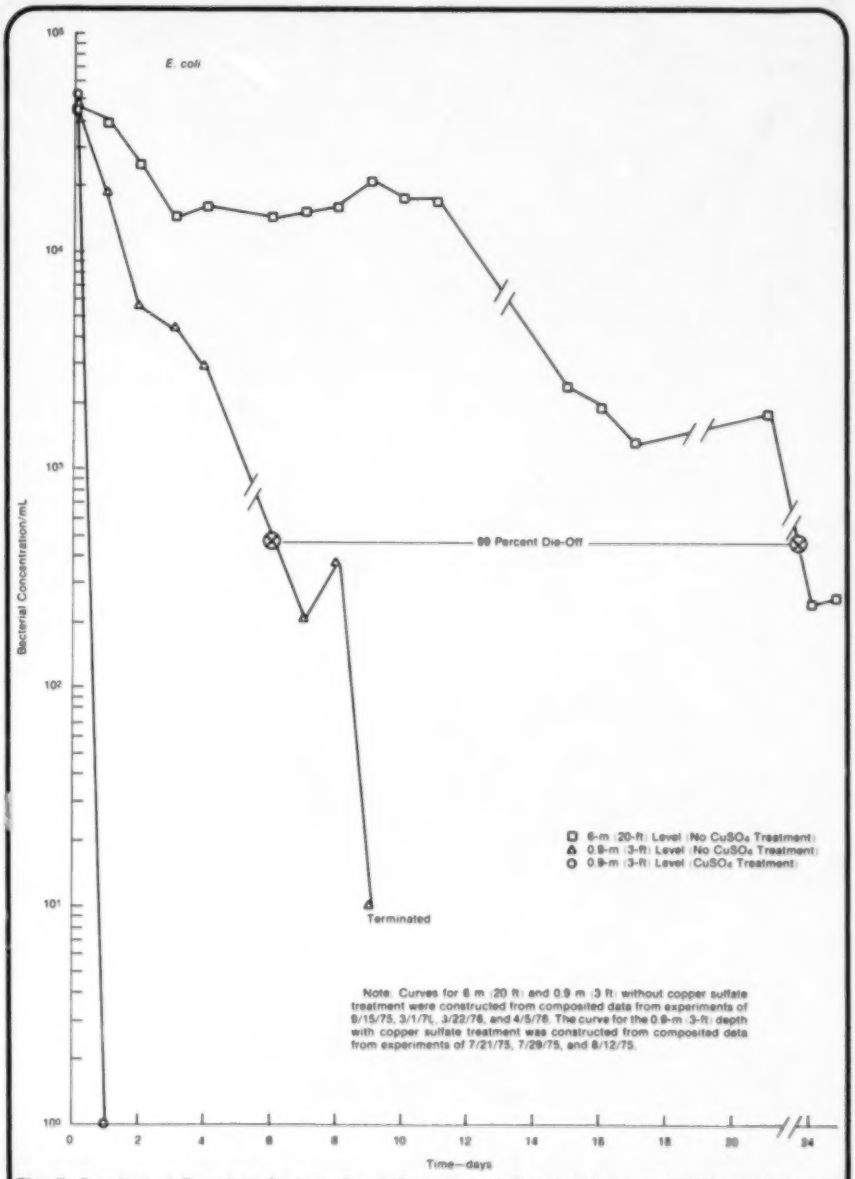
The negative results for *Salmonella* detection in the reservoir were not unexpected because the maximum fecal coliform densities at all sites during the *Salmonella* sampling periods were less than 230 organisms per 100 mL. A previous study of recreational lakes and stream impoundments suggested that a minimum density of 150 fecal coliforms per 100 mL of overlying water was necessary before *Salmonella* occurrence could be detected in the bottom sedi-

ments.[67] Furthermore, no *Salmonellae* were isolated from the overlying waters of recreational lakes and stream impoundments used for water supply that had low fecal coliform densities. One explanation for the unexpected and inconsistent detection of *Salmonella* in bottom muds from Cruises Creek reservoir is that the methodology for mud analyses was probably inadequate.

A total of thirteen individual bacterial survival experiments were initiated in the impoundment over a two-year period that included winter, spring, and summer seasons. Regardless of season, a remarkable similarity in persistence of a given organism was observed. Figures 6, 7, and 8 are composite survival curves for *Salmonella bern, E. coli,* and a fecal streptococcus isolate, respectively. Examination of these figures indicated the fecal streptococcus strain was undetectable within five days at the 0.9-m (3-ft) depth and within 21 days at a depth of 6.1 m (20 ft). Both *Salmonella bern* and *E. coli* had somewhat longer persistence and similar die-off rates. Survival of these three organisms was always longer at the depth of 6.1 m (20 ft). A higher concentration of nutrients, less sunlight penetration to 9.1 m (20 ft), and lower water temperatures were probably major factors in the extended persistence when contrasted to results from bacterial survival chambers submerged at a depth of 0.9 m (3 ft). Water temperature at the 6.1-m (20-ft) level ranged from 7°C to 12°C during these studies and averaged 4°C cooler than that observed at the 0.9-m (3-ft) depth. Apparently, reduced dissolved oxygen concentration in the bottom water was not detrimental to survival of these facultative aerobic bacteria.

Three applications of copper sulfate at two-week intervals between Jun. 10 and Jul. 15, 1975, were made to control algae blooms. Each treatment consisted of trailing a bag of copper sulfate crystals behind a boat that spread 180 kg (400 lb) of the material around the periphery of the reservoir. Assuming uniform distribution, the concentration of active ingredient was calculated to be 1.7 mg/L. However, since the application was made at the perimeter of the reservoir, the initial copper sulfate concentration in these quiet waters could easily have exceeded by ten times the concentration in the deeper, central portions of the lake because of wind currents over the lake and slow diffusion into the deeper water. Analysis for copper sulfate in the reservoir water nine months later indicated concentrations ranging from 0.29 to 0.55 mg/L at the 6.1-m (20-ft) level.

The application of copper sulfate had a pronounced and immediate effect on bacteria in chambers suspended at the 0.9-m (3-ft) level. Die-off of all the organisms exposed was rapid, in most cases occurring in less than 24 hr and generally not exceeding 48 hr. Comparisons of the survival of organisms suspended at 0.9 m (3 ft) and at 6.1 m (20 ft) could not be made because the chambers were not placed at 6.1-m (20-ft) depths during this portion of the study. However, the 0.9-m (3-ft) depth survival study results were consistent with the indicator organism die-off in water samples collected at the reservoir surface immediately after copper sulfate was added (Table 4).


E. coli
99 Percent Die-Off
Terminated
6-m (20-ft) Level (No $CuSO_4$ Treatment)
0.9-m (3-ft) Level (No $CuSO_4$ Treatment)
0.9-m (3-ft) Level ($CuSO_4$ Treatment)
Note: Curves for 6 m (20 ft) and 0.9 m (3 ft) without copper sulfate treatment were constructed from composited data from experiments of 9/15/75, 3/1/76, 3/22/76, and 4/5/76. The curve for the 0.9-m (3-ft) depth with copper sulfate treatment was constructed from composited data from experiments of 7/21/75, 7/29/75, and 8/12/75.
Bacterial Concentration/mL
Time—days
Fig. 7. Survival of *E. coli* in Cruises Creek Reservoir at Depths of 6.1 m (20 ft) and 0.9 m (3 ft)

Summary

The microbiological quality of reservoirs and lakes fluctuates as a result of a variety of environmental forces that may alter the natural self-purification processes and lead to irreversible ecological changes. Adequate appraisal of the status of stored water quality and the detection of subtle ecological changes that may be occurring requires a microbiological sampling protocol that is sensitive to the three-dimensional aspects of a large body of water. The sampling protocol must include a vertical profile of the water column to reveal zones of thermal and microbial stratification. A well-designed horizontal grid sampling pattern will identify surface inputs of differing water qualities from feeder streams, stormwater runoff, and point source pollution. In situ measurements are neces-

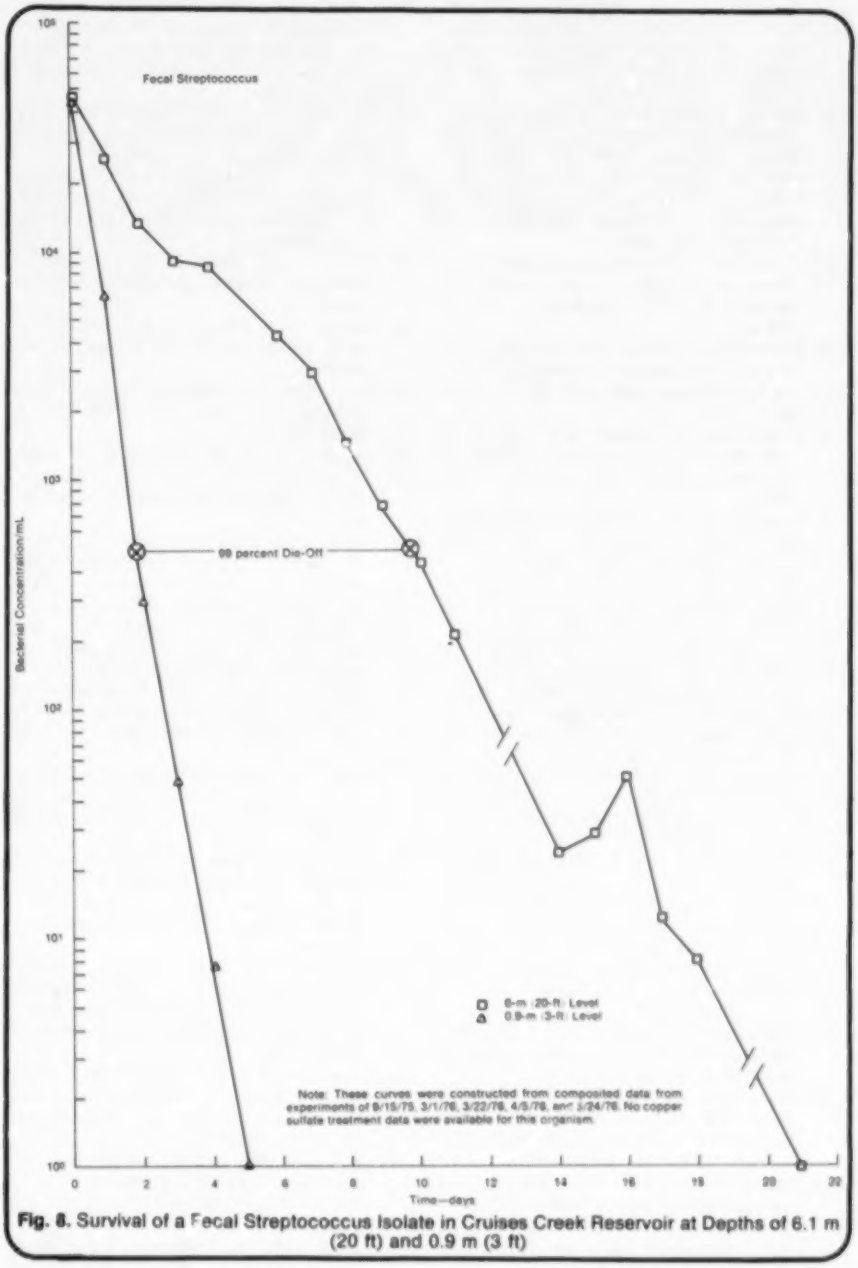

Fecal Streptococcus
99 percent Die-Off
6-m (20-ft) Level
0.9-m (3-ft) Level
Note: These curves were constructed from composited data from experiments of 9/15/75, 3/1/76, 3/22/76, 4/5/76, and 5/24/76. No copper sulfate treatment data were available for this organism.
Bacterial Concentration/mL
Time—days

Fig. 8. Survival of a Fecal Streptococcus Isolate in Cruises Creek Reservoir at Depths of 6.1 m (20 ft) and 0.9 m (3 ft)

sary for a realistic survival determination of the indicator organisms or salmonellae isolated from the reservoir or lake. Sample collection at the sediment-water interface is important as a measurement of the general quality of the overlying water, particularly where there is great variability in the bacterial quality of the water. Data from this type of sampling program on a source water reservoir revealed several factors that could help protect source water quality and reduce water treatment problems.

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References

1. Statistical Summary of Municipal Water Facilities in United States. PHS Pub. 1039 (Jan. 1, 1963).
2. Symons, J.M.; Carswell, J.K.; & Robeck, G.G. Mixing of Water Supply Reservoirs for Quality Control. *Jour. AWWA*, 62:5:322 (May 1970).
3. Dzyuban, A.N. The Number and Generation Time of Bacteria and Production of Bacterial Biomass in Water of the Saratov Reservoir. *Gidrobiol Zh.*, 11:14 (1975).
4. Romaninko, V.I. Total Bacterial Number in Rybinsk Reservoir. *Microbiologia* [USSR], 40:707 (1971).
5. Stepanek, M. Relation of Phytoplankton to the Bacterial Colonization of the Sedlice Reservoir. *Mitt. Int. Verein. Theor. Angero. Limnol.*, 14:201 (1968).
6. Niewolak, S. Microbiological Decomposition of Tribasic Calcium Phosphate in the Ilawa Lakes. *Acta Hydrobiol.*, 13:131 (1971).
7. Harrison, M.J. et al. Solubilization of Inorganic Phosphate by Bacteria Isolated from Upper Klamath Lake Sediment. *Limnol. Oceanogr.*, 17:50 (1972).
8. Niewolak, S. & Korycka, A. Solubilization of Basic-Slag by Microorganisms in Fertilized Lakes. *Polskie Archiwum Hydrobiol.*, 23:25 (1976).
9. Horvath, K. et al. Studies on the Water Quality of the Rohod-Reservoir (N.E. Hungary). *Acta Biol. Debrecina*, 12:119 (1975).
10. Yazar, O. et al. Effects of Selected Herbicides on Bacterial Populations in Non-Treated and Treated Water. *Water Resources Bull.*, 11:294 (1975).
11. Olivieri, V.P. et al. Microorganisms in Stormwater. EPA-600/2-77-087. USEPA, Cincinnati, Ohio (1977).
12. Geldreich, E.E. Buffalo Lake Recreational Water Quality: A Study in Bacteriological Data Interpretation. *Water Res.*, 6:913 (1972).
13. Gaufin, A.L. A Study of Natural Water Systems in Western Montana. Montana Univ. Joint Water Resources Res. Center, OWRT A-017-Mont. (1), Bozeman, Mont. (1974).
14. Reid, L.W. Wastewater Pollution and General Eutrophication of a Hydroelectric Impoundment. *Jour. WPCF*, 38:165 (1966).
15. King, J.G. & Mace, A.C. Jr. Effects of Recreation on Water Quality. *Jour. WPCF*, 46:2453 (1974).
16. Lee, R.E.; Symons, J.M.; & Robeck, G.G. Watershed Human-Use Level and Water Quality. *Jour. AWWA*, 62:7:412 (Jul. 1970).
17. Recreation on Water Supply Reservoirs, A Handbook for Increased Use. USGPO S/N 041-011-00027-1. Council on Environmental Quality, Washington, D.C. (1975).
18. Hibler, C.P.; MacLead, K.; & Lyman, D.O. Giardiasis—In Residents of Rome, N.Y., and in U.S. Travelers to the Soviet Union. *Morbid. Mortal. Weekly Rpt.*, 24:366 (1975).
19. Allard, J. et al. Waterborne Giardiasis Outbreaks—Washington, New Hampshire. *Morbid. Mortal. Weekly Rpt.*, 26:169 (1977).
20. Kirner, J.C.; Littler, J.D.; & Angelo, L. Giardiasis in Camas, Washington. *Jour. AWWA*, 70:1:35 (Jan. 1978).
21. Alter, A.J. Appearance of Intestinal Wastes in Surface Water Supplies at Ketchikan, Alaska. Proc. Fifth Alaska Sci. Conf. AAAS, Anchorage, Aka. (Sep. 1954).
22. Ketchikan Laboratory Studies Disclose Gulls are Implicated in Disease Spread.

Alaska's Health, 11:1 (1954).
23. Fennell, H.; James, D.B.; & Morris, J. Pollution of a Storage Reservoir by Roosting Gulls. *Water Treat. Exam.*, 23:5 (1974).
24. Menon, A.S. et al. Preliminary Bacteriological Investigation of the Lake Ontario Thermal Bar. Proc., Fourteenth Conf. on Great Lakes Res., Univ. of Toronto, Toronto, Ont. (1971).
25. Asthana, J.B. & Burdick, J.C. III. Bacteriological Water Quality Characteristics of Fort Loudon Reservoir. Tenn. Water Resources Res. Center. Res. Rpt. 21. OWRR A-013-Tenn. (4), 14-31-0001-3243 (1972).
26. Guthrie, R.K. et al. A Comparison of Thermal Loading Effects On Bacterial Populations in Polluted and Non-Polluted Aquatic Systems. *Water Res.* [G.B.], 8:143 (1974).
27. Bernhardt, H. & Wilhelms, A. The Pollution of a Reservoir by Inflowing Bacteria and Their Degradation. *Gas-u. Wass Fach (Wass. Abwass.)* [Ger.], 113:303 (1972).
28. Coutant, C.C. & Shapple, R.F. Bacteria in an Impounding Reservoir. *Jour. AWWA*, 58:10:1275 (Oct. 1966).
29. McLean, D.M.; Brown, J.R.; & Laak, R. Virus Dispersal by Water. *Jour. AWWA*, 58:8:920 (Aug. 1966).
30. Symons, J.M. Water Quality Behavior in Reservoirs. PHS Pub. 1930. Cincinnati, Ohio (1969).
31. Purcell, L.T. & Capen, C.H. North Jersey District Water Supply Commission Report on Treatment Plant and Transmission Main for Water Supply from Round Valley-Spruce Run Project for Participating Municipalities. New Jersey District Water Supply Commission, Wanaque, N.J. (1961).
32. Ridley, J.E. & Symons, J.M. New Approaches to Water Quality Control in Impoundments. *Microbiology of Polluted Waters* (R. Mitchell, editor). Wiley-Interscience, New York (1972).
33. Walesh, S.G. Natural Processes and Their Influence on Reservoir Water Quality Control. *Jour. AWWA*, 59:1:63 (Jan. 1967).
34. Bernhardt, H. Aeration of Wahnbach Reservoir Without Changing the Temperature Profile. *Jour. AWWA*, 59:8:943 (Aug. 1967).
35. Tortoriella, R.C. Manganic Oxide Reduction by Microorganisms in Freshwater Environments. *Dissertation Abs.*, 32:B:3408 (1971); *Water Poll. Abs.* [G.B.], 44:2602 (1971).
36. Inatin, A.V. The Number of Bacteria in the Water of Kuikyshev Reservoir. *Microbiologia*, 42:525 (1973).
37. Fry, J.C. et al. Changes in the Microbial Populations of a Reservoir Treated with Herbicide Paraquat. *Water Res.*, 7:395 (1973).
38. Sagidullaev, N.S. & Novoshilova, M.I. Heterotrophic Microflora of Ainu Darya Reservoirs and Its Role in Mineralization of Organic Matter. *Microbiologia* [USSR], 40:714 (1971).
39. Thurner, K. & Busse, M. Bacteriological Studies on Gravel-Pit Lakes. *Arch. Hydrobiol.*, 72:253 (1973).
40. Deufel, J. On the Transmission of Enterococci in Lake Constance. *Jour. Gas. u. Wass. Fach.* [Ger.], 112:442 (1971).
41. Bishop, R.A. & Lee, R.A. Spokane River, Cooperative Water Quality Study. Washington State Dept. of Ecology, 72-001. Olympia, Wash. (1972).
42. Novel, E. & Ranchi, W. Do the Waters of Lake Geneva Receive Pollution from Airborne Microflora. *Schweiz. Zeits. Hydrol.* [Switzerland], 30:381 (1969).
43. Geldreich, E.E. et al. The Bacteriological Aspects of Stormwater Pollution. *Jour. WPCF*, 40:1861 (1968).
44. Niewolak, S. Distribution of Microorganisms in the Waters of the Kortowskie Lake. *Pol. Arch. Hydrobiol.*, 21:315 (1974).
45. Drury, D.D. & Gearheart, R.A. Bacterial Population Dynamics and Dissolved Oxygen Minimum. *Jour. AWWA*, 67:3:154 (Mar. 1975).
46. Weiss, C.M. & Oglesby, R.T. Limnology and Water Quality of Raw Water in Impoundments. *Public Works*, 91:97 (1960).
47. Collins, V.G. The Distribution and Ecology of Bacteria. *Proc. Soc. Water Treat. Exam.* [England], 12:40 (1963).
48. Tsernoglou, D. & Anthony, E.H. Particle Size, Water-Stable Aggregates, and Bacterial Populations in Lake Sediments. *Can. Jour. Microbiol.*, 17:217 (1971).
49. Niewolak, S. Microbiological Characterization of Ground Deposits in the Lakes of the District of Ilawa in the Years 1960-1963. *Zeszyty Naukowe Myzszej Szkoly Rolniczej w Olsztynie*, 28:613 (1970).
50. Boylen, C.W. & Brock, T.O. Bacterial Decomposition Processes in Lake Wingra Sediments During Winter. *Limnol. Oceanogr.*, 18:628 (1973).
51. Hussenot, J. & Laurent, M. Evaluation of Bacterial Production in a Pond in Cologne. *Amn. Hydrogiol.*, 4:169 (1973).
52. Novozhilova, M.I. & Berezina, F.S. Character of the Distribution of Sulfate-Reducing and Sulphur Bacteria in the Sediments of Lake Balkhash. *Mikrobiologia*, 37:436 (1968).
53. Aliverdieva-Gamidova, L.A. Microbiological Processes in Lake Mekhteb. *Mikrobiologia*, 38:943 (1969).
54. Kevin, M.A. & Brezonik, P.L. Nitrogen Fixation by Bacteria in Lake Mize, Florida, and in Some Lacustrine Sediments. *Limnol. Oceanogr.*, 16:720 (1971).
55. Yarushek, N.E. Quantity of Bacteria and Destruction of Organic Matter in Bottom Deposits of the Saratov Water Storage Basin. *Gidrobiol. Zh. Akad. Nauk. Vkr.* [USSR], 9:83 (1973).
56. Dutka, B.J. et al. Microbiological Examination of Offshore Lake Erie Sediments. *Jour. Fish Res. Bd. Can.*, 31:299 (1974).
57. Fonden, R. The Distribution of Bacteria in Lake Hjalmaren 1965-1967. Inst. Limnol., Uppsala. Lake Malaren Res. Rept. 25 (1968).
58. Lighthart, B. A Cluster Analysis of Some Bacteria in the Water Column of Green Lake, Washington. *Can. Jour. Microbiol.*, 21:392 (1975).
59. Biswas, E. & Ruth, I. Changes in the Heterotrophic Bacteria of Volta Lake, 1968-1971. *Ghana Jour. Sci.*, 12:72 (1972).
60. Graf, W. & Bauer, L. Red Bacterial Growth (*Corynebacterium rubrum* n. Spec.) in Tap Water Systems. *Zbl. Bakt. Hyg., I. Abt. Orig. B.*, 157:291 (1973).
61. Truper, H.G. & Genovese, S. Characterization of Photosynthetic Sulphur Bacteria Causing Red Water in Lake Faro (Messina, Sicily). *Limnol. Oceanogr.*, 13:225 (1968).
62. Bowers, L. & Bishop, W. Bacterial Ecology of A Meromictic Lake. *Verh. Intl. Ver. Limnol.* [Ger.], 16:1501 (1966).
63. Alhonen, S.M. On the Identification and Importance of Purple Bacteria Occurring in Lake Sediments. *Vesitalous* [Fin.], 11:15 (1970).
64. Rodina, A.G. Bacterial Population of Humified Lakes. *Microbiol.*, 38:448 (1969).
65. Schegg, E. A New Bacteriological Sampling Bottle. *Limnol. Oceanogr.*, 15:820 (1970).
66. Joyce, J.R. An Improved Bottom-Water Sampler. *Jour. Mar. Biol. Assn.* [U.K.], 53:741 (1973).
67. Van Donsel, D.J. & Geldreich, E.E. Relationships of *Salmonellae* to Fecal Coliforms in Bottom Sediments. *Water Res.*, 5:1079 (1971).
68. Van Donsel, D.J. & Geldreich, E.E. Bacterial Bottom Sampler for Water Sediments. *The Aquatic Environment: Microbial Transformations and Water Management Implications* (L.J. Guarraia and R.K. Ballentine, editors) EPA 430/G-73-008. USEPA, Washington, D.C. (1972).
69. *Standard Methods for the Examination of Water and Wastewater*. APHA, AWWA, and WPCF, Washington, D.C. (13th ed., 1971).
70. Spino, D.F. Elevated-Temperature Technique for the Isolation of *Salmonella* from Streams. *Appl. Microbiol.*, 14:591 (1966).
71. Hajna, A.A. A Proposed Rapid Method of Differentiating and Identifying Bacteria of the Intestinal Group in State Public Health Laboratories. *Pub. Health Lab.*, 9:23 (1951).
72. McFeters, G.A. & Stuart, D.G. Survival of Coliform Bacteria in Natural Waters: Field and Laboratory Studies with Membrane Filter Chambers. *Appl. Microbiol.*, 24:805 (1972).
73. Fliermans, C.B. & Gorden, R.W. Modification of Membrane Diffusion Chambers for Deep-Water Studies. *Appl. Envir. Microbiol.*, 33:207 (1977).
74. Churchill, M.A. Effects of Storage Impoundments on Water Quality. Paper 2928. *Transactions-ASCE*, 123:419 (1958).
75. Powell, M.P. & Berthouex, P.M. Water Quality Changes Due to Impoundment. *Jour. AWWA*, 59:7:829 (Jul. 1967).
76. Geldreich, E.E. Fecal Coliform and Fecal Streptococcus Density Relationships in Waste Discharge and Receiving Waters. *CRC Critical Reviews in Envir. Contr.*, 6:349 (1976).

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